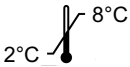
ViennaLab®

CAH StripAssay®

Instructions For Use

REF	Σ
4-380	20 tests
4-380-A	48 tests
4-380-TRIAL	5 tests



IVD

CE 0123

Version: rev 1.0 / English
eIFU and other languages available at
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REVISION HISTORY:

version	date	description
rev 1.0	2022-11	Addition of IVDR-related contents to version 2022-01.

Summary of Safety and Performance (SSP) of the StripAssay® is retrievable from the European Database on Medical Devices (EUDAMED): https://ec.europa.eu/tools/eudamed or from the manufacturer.

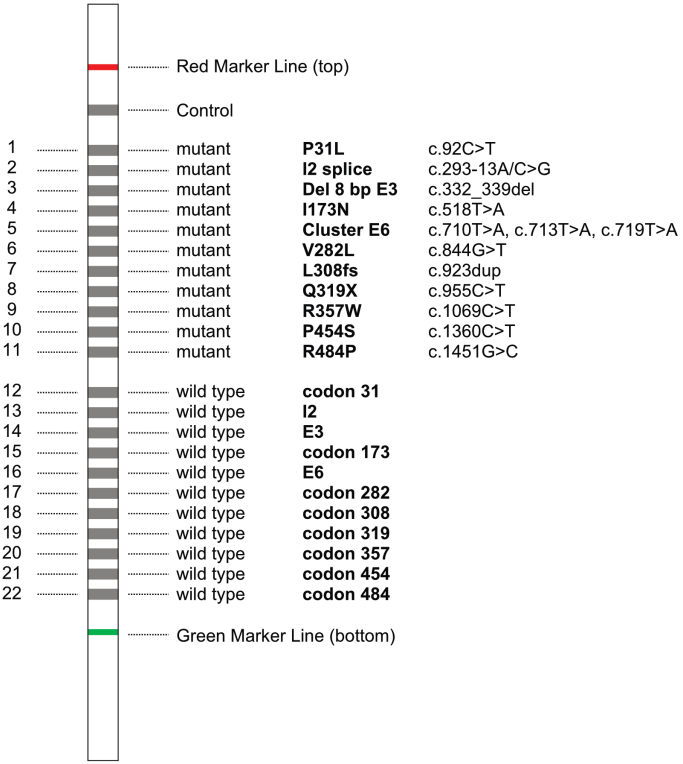

Fig. 1: Teststrip Design

Note: Teststrips are not drawn in real size and must not be used for interpretation of results!

I. INTENDED PURPOSE

The CAH StripAssay® is a qualitative genetic test for the targeted analysis of 11 common mutations in the *CYP21A2* gene causing congenital adrenal hyperplasia (CAH). Mutation analysis is performed on DNA extracted from peripheral blood samples or from dried blood spots. The test is used as an aid to confirm the presence of *CYP21A2* mutations in patients with a suspected diagnosis of CAH and as a second-tier diagnostic test in newborn screening programs. The StripAssay® can be carried out either manually or semi-automated.

For human *in vitro* diagnostic use.

II. BACKGROUND

CAH is an autosomal recessive disorder of the adrenal cortex with an incidence of 1:10,000-20,000. In about 95% of cases the condition is caused by molecular defects in the steroid 21-hydroxylase gene *CYP21A2*. The resulting disorder of adrenal steroid metabolism is characterized by a lack of cortisol and aldosterone biosynthesis. Decrease of cortisol prompts corticotropin (ACTH) stimulation of the adrenal cortex and results in overproduction of steroid precursors. Some of these precursors are diverted to sex hormone biosynthesis, leading to androgen excess starting from early intrauterine life. The phenotype of CAH correlates with the severity of *CYP21A2* mutations and includes classic salt-wasting CAH (SW-CAH), classic simple-virilizing CAH (SV-CAH), as well as mild non-classic forms of the disease (NC-CAH). Females (46,XX) show various degrees of genital virilization at birth, whereas males (46,XY) may appear inconspicuous. Neonatal CAH-screening based on the assessment of 17-hydroxyprogesterone levels has been introduced in many countries, but also has a high false positive rate. Therefore, the implementation of genetic second-tier tests is an aid for diagnosis and can significantly reduce recall rates.

Important!

The CAH StripAssay® covers common single nucleotide variants and a small deletion in the *CYP21A2* gene. Twenty to 25% of pathogenic variants are large *CYP21A2* deletions, duplications or chimeric genes. Therefore, assessment of the *CYP21A2* copy number variation (CNV) is important for diagnosis. The combination of both, ViennaLab CAH StripAssay® and CAH RealFast™ CNV Assay allows for a targeted analysis of the *CYP21A2* gene with a detection rate of up to 94%.

III. METHODOLOGY

The CAH StripAssay® is based on polymerase chain reaction (PCR) and reverse-hybridization. The procedure includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to a Teststrip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines (Fig. 1). Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

The CAH StripAssay® detects the following mutations in the *CYP21A2* gene locus:

	legacy name		**HGVS nomenclature**		**RefSNP**
	new	old			
1	P31L	(P30L)	c.92C>T	p.Pro31Leu	rs9378251
2	I2 splice	I2 splice	c.293-13A/C>G	splicing defect	rs6467
3	Del 8 bp E3	Del 8 bp E3	c.332_339del	p.Gly111fs	rs387906510
4	I173N	(I172N)	c.518T>A	p.Ile173Asn	rs6475
5	Cluster E6 I237N	(I236N)	c.710T>A	p.Ile237Asn	rs111647200
	Cluster E6 V238E	(V237E)	c.713T>A	p.Val238Glu	rs12530380
	Cluster E6 M240K	(M239K)	c.719T>A	p.Met240Lys	rs6476
6	V282L	(V281L)	c.844G>T	p.Val282Leu	rs6471
7	L308fs	(L307fs)	c.923dup	p.Leu308fs	rs267606756
8	Q319X	(Q318X)	c.955C>T	p.Gln319Ter	rs7755898
9	R357W	(R356W)	c.1069C>T	p.Arg357Trp	rs7769409
10	P454S	(P453S)	c.1360C>T	p.Pro454Ser	rs6445
11	R484P	(R483P)	c.1451G>C	p.Arg484Pro	rs200005406

Reference Sequence (RefSeq):

NM_000500.9
NP_000491.4

The test can be carried out manually or semi-automated using instruments designed for automation of Teststrip processing (see section VI. 3.4).

IV. KIT COMPONENTS

REF

	4-380	4-380-A	4-380 -TRIAL
1. **Lysis Solution**	50 ml	---	50 ml
2. **GEN^{X}TRACT™ Resin**	5 ml	---	5 ml
3a. **Amplification Mix A** *(yellow cap)*	500 µl	2x 500 µl	500 µl
3b. **Amplification Mix B** *(white cap)*	500 µl	2x 500 µl	500 µl
3c. **Amplification Mix C** *(green cap)*	500 µl	2x 500 µl	500 µl
4. **Taq Dilution Buffer** *(transparent cap)*	500 µl	2x 500 µl	500 µl
5. **HS-Taq DNA Polymerase (5 U/µl)** *(red cap)*	175 U	2x 175 U	125 U
6. **DNAT** *(blue cap)*	1.5 ml	2x 1.5 ml	1.5 ml
⚠ Warning: DNAT contains 1.6 % NaOH H315: Causes skin irritation H319: Causes serious eye irritation P280: Wear protective gloves/protective clothing/eye protection/face protection P337 + P313: If eye irritation persists: Get medical advice/attention			
7. **Typing Trays**	3	---	1
8. **Teststrips**	20	2x 24	5
9. **Hybridization Buffer** *(white cap)*	25 ml	65 ml	25 ml
10. **Wash Solution A** *(white cap)*	80 ml	200 ml	80 ml
11. **Conjugate Solution** *(transparent cap)*	25 ml	65 ml	25 ml
12. **Wash Solution B** *(transparent cap)*	80 ml	200 ml	80 ml
13. **Color Developer** *(brown cap)*	25 ml	65 ml	25 ml
⚠ Warning: Color Developer contains ≤0.4% maleic acid H317: May cause an allergic skin reaction P280: Wear protective gloves/protective clothing/eye protection/face protection P302 + P352: If on skin: wash with plenty of water P333 + P313: If skin irritation or rash occurs: get medical advice /attention			
14. **Instructions For Use**	1	1	1
15. **Collector™ Sheet**	1	3	1

Note: Store all reagents at 2°C to 8°C when not in use!

name of component	composition
Lysis Solution	hypotonic solution containing $KHCO_3$, NH_4Cl, EDTA
GEN^{X}TRACT™ Resin	Chelex 100 Resin MB in a buffered solution
Amplification Mix A	sequence-specific 5'-biotin labelled oligonucleotides, an equimolar mixture of deoxy ribonucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), ammonium sulfate buffer, glycerol, 0.05% sodium azide
Amplification Mix B and C	sequence-specific 5'-biotin labelled oligonucleotides, an equimolar mixture of deoxy ribonucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), $(NH_4)_2SO_4$, KCl, 0.05% sodium azide
Taq Dilution Buffer	buffer for HS-Taq DNA Polymerase, including KCl, $(NH_4)_2SO_4$ and $MgCl_2$, 0.05% sodium azide

name of component	composition
HS-Taq DNA Polymerase (5 U/µl)	hot-start-Taq DNA polymerase at a concentration of 5U/µl
DNAT	basic solution containing 1.6 % sodium hydroxide and a blue dye indicating a change of pH
Typing Trays	plastic tray with eight wells
Teststrips	allele-specific oligonucleotide probes and a hybridization control immobilized as an array of parallel lines on a polyester-supported membrane framed by a red line on the top and a green line on the bottom
Hybridization Buffer	phosphate buffer with <2% detergent
Wash Solution A	citrate buffer with <1% detergent
Conjugate Solution	streptavidin conjugated alkaline phosphatase diluted in a saline based buffer with 0.05% sodium azide
Wash Solution B	tris buffer containing <2% detergent and 0.05% sodium azide
Color Developer	color substrate for the alkaline phosphatase contains nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP)
Instructions For Use	printed paper
Collector™ Sheet	printed paper

V. MATERIALS REQUIRED BUT NOT SUPPLIED

In addition to standard molecular biology laboratory equipment, the following is needed:

- Thermoblock or thermomixer for 1.5 ml reaction tubes with temperature control up to 99°C
- Adjustable microcentrifuge capable of 3,000-12,000 rpm (1,000-12,000 x g)
- Thermocycler with heated lid (for specification of ramp rates see section VIII)
- Waterbath with shaking platform, lid and adjustable temperature (45°C ± 1°C)
- Shaker (rocker or orbital shaker)

Optional:

- Vacuum aspiration apparatus
- Thermoshaker for microtiter plate format with lid and adjustable temperature (45°C ± 1°C), e.g. PST-60 HL (Biosan) or equivalent device
- Instrument for automated hybridization, adjustable to the time-temperature profile as described in section VI. 3.4, e.g. DYNABLOT Heat (Dynex) or equivalent device
- Agarose gel electrophoresis equipment (for control of amplification products)

VI. ASSAY PROCEDURE

1. Sample Preparation

1.1. DNA-Extraction from EDTA-anticoagulated Blood

Use fresh or frozen blood with EDTA anticoagulant. Blood containing heparin or citrate has not been tested. Do not store blood for more than 3 days at ambient temperature or more than 1 week at 2°C to 8°C before use. Blood that has been kept frozen for more than one year, or gone through more than three freeze-thaw cycles shall not be used. For specimen collection and transportation follow the instructions for use of the EDTA-blood collection tube and general recommendations for blood sampling.

Bring blood samples to room temperature. Mix well by carefully inverting blood collection tubes several times. Allow Lysis Solution and GENXTRACT™ Resin to reach room temperature.

- Pipette **100 µl blood sample** into a 1.5 ml microtube with screw cap.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Let stand for **15 min.** at room temperature.
- Centrifuge for **5 min.** at **3,000 rpm** (approx. 1,000 x g) in a microcentrifuge.
- Remove and discard the upper (top) 1 ml of supernatant.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Centrifuge for **5 min.** at **12,000 rpm** (approx. 12,000 x g) in a microcentrifuge.
- Remove and discard the supernatant except for approx. 50 µl of a visible, soft pellet.
- Resuspend GENXTRACT™ Resin by swirling the bottle thoroughly.
- Add **200 µl GENXTRACT™ Resin** to the pellet. Close tube and vortex for 10 sec.

Note: GENXTRACT™ Resin sediments quickly. Repeat resuspension each time immediately before removing another aliquot.

- Incubate for **20 min.** at **56°C**. Vortex for 10 sec.
- Incubate for **10 min.** at **98°C**. Vortex for 10 sec.
- Centrifuge for **5 min.** at **12,000 rpm** in a microcentrifuge. Cool on ice.

The resulting supernatant contains DNA template suitable for immediate use in PCR. For further storage, the supernatant should be transferred into a fresh tube and kept refrigerated (2°C to 8°C; up to one week) or frozen at -30°C to -15°C (for long term).

Use of other DNA isolation methods with the CAH StripAssay® has not been validated. In case other DNA extraction systems are used, concentration and purity of DNA should be within a range of 2 to 10 ng/µl and an $OD_{A260/280}$ ratio of 1.7 to 2.0, respectively. Higher DNA concentrations have to be diluted to the recommended range prior to PCR input.

1.2. DNA Extraction from Dried Blood Spots (DBS)

By using a lancet collect blood drops from a finger or heel prick onto an absorbent filter paper suitable for neonatal screening (Whatman 903 Protein Saver or PerkinElmer 226 filter papers). Allow the blood spot to air-dry for maximum 24 hours at room temperature. For long term archiving up to one year, store DBS at 2°C to 8°C in a resealable bag with a desiccant pouch.

Allow Lysis Solution and GENXTRACT™ Resin to reach room temperature.

- Place **two 3 mm punches of a DBS** into a 1.5 ml microtube with screw cap.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.

- Let stand for **10 min.** at room temperature.
- Centrifuge for **1 min.** at **12,000 rpm** (approx. 12,000 x g) in a microcentrifuge.
- Remove and discard the supernatant completely.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Let stand for **10 min.** at room temperature and mix by inverting several times.
- Centrifuge for **1 min.** at **12,000 rpm** in a microcentrifuge.
- Remove and discard the supernatant completely.
- Resuspend GENXTRACT™ Resin by swirling the bottle thoroughly.
- Add **200 µl GENXTRACT™ Resin** to the punches. Close tube and mix gently by tapping the bottom of the tube. Punches should be completely submerged by the resin.

Note: GENXTRACT™ Resin sediments quickly. Repeat resuspension each time immediately before removing another aliquot.

- Incubate for **20 min.** at **56°C**.
- Incubate for **10 min.** at **98°C**.
- Centrifuge for **5 min.** at **12,000 rpm** in a microcentrifuge. Cool on ice.

The resulting supernatant contains DNA template suitable for immediate use in PCR. For further storage, the supernatant should be transferred into a fresh tube and kept refrigerated (2°C to 8°C; up to one week) or frozen at -30°C to -15°C (for long term).

2. In Vitro Amplification (PCR) – 3 separate reactions per sample

Important: Keep all PCR reagents and DNA templates refrigerated throughout.

- Freshly prepare each time an appropriate amount of working solution (1:20, final conc. 0.25 U/µl) of **HS-Taq DNA Polymerase** (5 U/µl, red cap) in **Taq Dilution Buffer** (transparent cap) for the number of samples to be analyzed, plus the **no-template control**.

component	per reaction	e.g. 10 reactions
HS-Taq DNA Polymerase (5 U/µl)	0.25 µl	2.5 µl
Taq Dilution Buffer	4.75 µl	47.5 µl
working solution	5 µl	50 µl

- Prepare three reaction tubes for each sample to be amplified. Place tubes on ice.
- For each sample prepare 3 final PCR reaction mixes (A, B, C) on ice:

A: **15 µl Amplification Mix A** (yellow cap)
5 µl diluted HS-Taq DNA Polymerase (1.25 U)
5 µl DNA template

B: **15 µl Amplification Mix B** (white cap)
5 µl diluted HS-Taq DNA Polymerase (1.25 U)
5 µl DNA template

C: **15 µl Amplification Mix C** (green cap)
5 µl diluted HS-Taq DNA Polymerase (1.25 U)
5 µl DNA template

Note: It is recommended to prepare a mastermix for all samples containing Ampification Mix and diluted HS-Taq DNA Polymerase. First pipette 20 µl of the mastermix into each PCR tube, and then add DNA template. Include a no-template control in each run by using PCR grade water instead of DNA (or preferably the negative control of your DNA extraction).

Generally, prepare working solutions / mastermix with a 10% excess volume to compensate for pipetting inaccuracies.

- Cap tubes tightly. Preheat the thermocycler to 95°C.
- Insert reaction tubes and run the following thermocycling program:

pre-PCR: 95°C/2 min.
thermocycling: 95°C/30 sec. - 62°C/30 sec. - 72°C/2:30 min. (40 cycles)
final extension: 72°C/7 min.

- Store amplification products on ice or at 2°C to 8°C for further use.

Optional: Analyze amplification products by gel electrophoresis (e.g. 3% agarose gel).

Fragment lengths: 1460 bp (A)
2071 bp (B)
1675 bp (C)

3. Processing of Teststrips

3.1. Hybridization (manual) – 1 Teststrip per sample (45°C, shaking waterbath)

Important: Adjust the water level of the waterbath to approx. ½ of the height of the Typing Tray. Heat the waterbath to exactly 45°C. Check water temperature with a calibrated thermometer. Prewarm Hybridization Buffer and Wash Solution A to 45°C. Take care that all precipitates formed at 2°C to 8°C become completely dissolved. Allow Teststrips, DNAT, Conjugate Solution, Wash Solution B and Color Developer to reach room temperature. Prepare Typing Tray(s).

Remove one Teststrip for each sample using clean tweezers. Touch Teststrips with unpowdered gloves only! Label Teststrips outside of the marker lines with a pencil (no ballpoint pens, markers, etc.).

- Pipette **40 µl DNAT** (blue cap) into the lower corner of each lane to be used in the Typing Trays (one lane per sample).
- Add **20 µl amplification product A** into the corresponding drop of DNAT.
- Add **20 µl amplification product B** into the same drop.
- Add **20 µl amplification product C** into the same drop.
- Mix thoroughly with a pipette. (The solution will remain blue.)
- Let stand for **5 min.** at room temperature.
- Add **1 ml Hybridization Buffer** (prewarmed to 45°C) into each lane.
  Gently agitate tray. (The blue color will disappear.)
- Insert **Teststrips** with marked side up (lines visible!) into the respective lanes. Submerge completely.
- Incubate for **30 min.** at **45°C** on the shaking platform of the waterbath.

Set moderate shaking frequency (approx. 50 rpm) to avoid spilling. Keep the cover of the waterbath closed to avoid variations in temperature.

- At the end of incubation remove hybridization solutions by vacuum aspiration or pipetting.

Proceed immediately. Do not allow Teststrips to run dry during the entire procedure.

3.2. Stringent Wash (45°C, shaking waterbath)

- Add **1 ml Wash Solution A** (prewarmed to 45°C). Rinse briefly (10 sec.).
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution A** (45°C).
- Incubate for **15 min.** at **45°C** in the shaking waterbath.
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution A** (45°C).
- Incubate for **15 min.** at **45°C** in the shaking waterbath.
  Remove liquids by vacuum aspiration or pipetting.

3.3. Colorimetric Detection (room temperature, 22°C ± 3°C)

- Add **1 ml Conjugate Solution**.
- Incubate for **15 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution B**. Rinse briefly (10 sec.).
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution B**.
- Incubate for **5 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Wash Solution B**.
- Incubate for **5 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration or pipetting.
- Add **1 ml Color Developer**.
- Incubate for **15 min.** at **room temperature** in the dark on a rocker or orbital shaker.
  A purple staining will appear upon positive reaction.
- Wash Teststrips several times with distilled water.
  Let strips dry in the dark on absorbent paper.

Do not expose Teststrips to intense light after Color Development.

3.4. Hybridization (automated) - optional instead of waterbath and shaker

An instrument for the automated processing of Teststrips shall meet the following requirements:

- Programmable temperature and time profile according to section 3.1 to 3.3 of the StripAssay® procedure.
- Integrated preheating station for Hybridization Buffer and Wash Solution A.
- Temperature control of trays during Hybridization and Stringent Wash steps at 45°C ± 1°C.
- Active cooling system of the tray to ensure rapid temperature decrease for Colorimetric Detection steps at room temperature.
- Shaking capability for tray.
- Heated lid for the tray to avoid evaporation of reagents during incubation.
- Dispensation of defined reagent volumes.
- Aspiration of reagents.
- Depending on the instrument used and the number of samples processed in one run, additional reagents may be required. Separate StripAssay® Detection Reagents are available for 20 tests (REF CS-012) and 48 tests (REF CS-017).

VII. INTERPRETATION OF RESULTS

The genotype of a sample is determined using the enclosed Collector™ sheet. Place the processed Teststrip into the designated fields, align it to the schematic drawing using the red marker line (top) and the green marker line (bottom), and fix it with adhesive tape.

A positive reaction of the uppermost Control line indicates the correct function of Conjugate Solution and Color Developer. This line should always stain positive.

For each polymorphic position, one of the following staining patterns (Fig. 2) should be obtained:

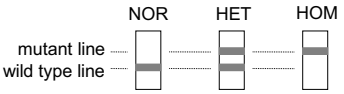

Fig. 2: Genotypes – staining patterns on the Teststrip

	wild type line	mutant line	genotype
NOR	**positive**	negative	normal
HET	**positive**	**positive**	heterozygous
HOM	negative	**positive**	homozygous mutant

Note: Staining intensities of positive lines may vary. This is of no significance for the result.

See examples of StripAssay® results on page 19 (Fig. 3).

Deletions and duplications of the *CYP21A2* gene, or large conversions between the functional gene and its highly homologous pseudogene (*CYP21A1P*) comprise about 30 percent of genetic alterations found in congenital adrenal hyperplasia (CAH) patients. It is therefore always recommended to combine the results of the CAH StripAssay® with data on *CYP21A2* gene copy number, obtained for example by the ViennaLab CAH RealFast™ CNV Assay (REF 7-410) or by Multiplex Ligation-dependent Probe Amplification (MLPA). In certain cases conversions may be indicated by unusual StripAssay® patterns (e.g. the common "30 kb E1-E3 deletion"; see examples E-H, page 19).

The CAH StripAssay® will not discriminate between a homozygous mutation (present on both alleles) and a hemizygous mutation (deletion present on second allele; see examples C, H and K, page 19).

To specifically amplify the functional *CYP21A2* gene, some primers had to be selected within potentially mutated regions. Therefore, the presence of the Cluster E6 mutation interferes with the amplification of the region spanning P31L and I2 splice. If one of these mutations is present in combination with Cluster E6, they will appear like being pseudo-homozygous (see example J, page 19). If Cluster E6 is present in the homozygous or hemizygous state, the wildtype signals for P31L and I2 splice will be missing (see example K, page 19).

As with any diagnostic test, results of the CAH StripAssay® shall be interpreted in the context of the patient's overall clinical phenotype and other medical investigations available to the physician. ViennaLab Diagnostics GmbH is not responsible for any clinical decisions that are taken.

VIII. PERFORMANCE EVALUATION

Accuracy of the CAH StripAssay® was determined by analyzing 271 pretyped genomic DNA samples. Results were completely concordant with the reference method Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA). The assay correctly detected one or more mutations in 201 samples (= 100% Positive Percent Agreement) and no mutation in 70 samples (= 100% Negative Percent Agreement).

Precision of the CAH StripAssay® was assessed as variability between replicates, operators, days, thermocyclers, hybridization devices, sample material (whole blood, dried blood spots) and DNA extraction kits. In a total of 78 tests carried out under the investigated parameters, all tests showed the expected genotyping results. Only negligible differences in staining intensity of Teststrips were visible, and no background staining was observed. The CAH StripAssay® was validated on the AB GeneAmp® PCR System 2700, MJ Research PTC-200 and Eppendorf Mastercycler X50s, which represent a heating and cooling rate in the range of 1.7 to 6.3°C/sec and 1.4 to 3.7°C/sec, respectively.

Use of other thermocyclers must be verified by the user.

Analytical Specificity is first and foremost ensured by the selection of the gene-specific primers and the allele-specific capture probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene databases by sequence comparison analysis. Thereby, the detectability of all relevant genotypes has been ensured. Potential cross-reactivity between capture probes was verified by synthetic DNA harboring the respective gene fragment. No cross-reactivity was observed.

Clinical Performance: Assessment of the clinical performance of the CAH StripAssay® in order to support clinical evidence comprised a systematic review of available data and applicable elements. As a result of the literature search, three publications were identified as pertaining to the safety and performance of the CAH StripAssay®, which demonstrate the clinical utility of the assay. Publications included a multicenter study reporting on the development and validation of the CAH StripAssay® on 271 patient samples (Németh et al., Clin Chim Acta. 2012 Dec 24;414:211-4). No adverse events or deviations have been identified within method comparison studies. In summary, the clinical performance, the benefits, and the safety of the CAH StripAssay® is confirmed when the device is used as intended for confirmatory diagnosis of congenital adrenal hyperplasia.

IX. INTERFERING SUBSTANCES

Five interfering substances (hemoglobin, immunoglobin G, traces of blood, ethanol and EDTA) potentially being present in EDTA-blood derived DNA preparations have been tested. Their effects on PCR were evaluated in three purified DNA samples spiked with various concentrations of substances and compared to their controls without addition of any interfering substances. All samples were analyzed in triplicate.

A final concentration of <10 µM hemoglobin, 0.1 µM immunoglobulin G, <1% peripheral blood, 1.25% ethanol or 0.1 mM EDTA in the reaction did not interfere with StripAssay® performance.

X. LIMITATIONS OF THE ASSAY

The CAH StripAssay® is exclusively designed for the diagnosis of 11 common mutations as listed in section III, which are represented by allele-specific capture probes on the Teststrips. Other *CYP21A2* point mutations, deletions or conversions that may be present in a patient's sample cannot be detected.

The assay is designed to amplify the entire *CYP21A2* gene in three overlapping fragments using biotinylated PCR primer pairs that do not co-amplify the highly homologous pseudogene *CYP21A1P*. Fragment A enfolds the region from exons 1 to 6, while fragments B and C from exons 3 to 10 and exons 5 to 10, respectively. Fusions of the active gene and the pseudogene, so called *CYP21A1P/CYP21A2* chimeras with junction sites downstream of Cluster E6, as well as complete *CYP21A2* deletions, cause a partial PCR dropout, and only the allele carrying a wild-type sequence or a point mutation is amplified. In the latter case, mutations appear to be pseudo-homozygous despite the real hemizygous condition. For a comprehensive analysis it is therefore necessary to determine the *CYP21A2* copy number variation using a complementary method (e.g. ViennaLab CAH RealFast™ CNV Assay).

Rare or private variants within primers and probes binding sites, as well as deletions or gene conversions may lead to amplification failure and missing signals on Teststrips.

The CAH StripAssay® must not be used for the purpose of prenatal diagnosis or preimplantation genetic diagnosis. The assay has not been validated on specimens derived from chorionic villus sampling, amniotic fluid or umbilical cord blood.

The CAH StripAssay® is intended for laboratory professional use only.

XI. QUALITY CONSIDERATIONS

- A thorough understanding of the procedure outlined here, as well as standard laboratory techniques and appropriate equipment are required to obtain reliable results.
- Do not use StripAssay® kits beyond their expiration date.
- After first opening of the primary container, StripAssay® reagents are stable until the expiry date printed on the outer label of the kit when stored properly at 2°C to 8°C.
- Use sterile disposable pipette tips with filters to avoid microbial contamination and cross-contamination of reagents or samples. Do not interchange bottle caps.
- Single use only.

XII. SAFETY

- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and disposable gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with clinical specimens in a biohazard waste container.
- Avoid contact of DNAT and Color Developer with skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. If spilled, dilute with water before wiping dry.
- Adhere to all local and federal safety and environmental regulations which may apply.

XIII. TECHNICAL SUPPORT

Technical support may be obtained by:

- the local ViennaLab Diagnostics distributor (www.viennalab.com/distribution)
- Video Tutorials (www.viennalab.com/support)
- the StripAssay® Manual (www.viennalab.com/support)
- the StripAssay® Troubleshooting Guide (www.viennalab.com/support)
- contacting techhelp@viennalab.com

XIV. REFERENCES

- OMIM Online Mendelian Inheritance in Man (www.omim.org)
- Németh et al. Clin Chim Acta 2012; 24;414:211-4

XV. FEEDBACK TO THE MANUFACTURER

Any serious incident that has occurred in relation to the StripAssay® must be reported to the competent authority of the country and to the manufacturer.

XVI. SYMBOLS

REF	Catalog number
LOT	Batch code
IVD	*In vitro* diagnostic medical device
CE 0123	Compliant with European IVD Regulation 2017/746 Identification number of notified body
	Sufficient for <n> tests
	Storage temperature limits
	Use by
	Caution
	Manufacturer
	Date of manufacture
	Consult Instructions For Use

XVII. EXAMPLES OF TEST RESULTS

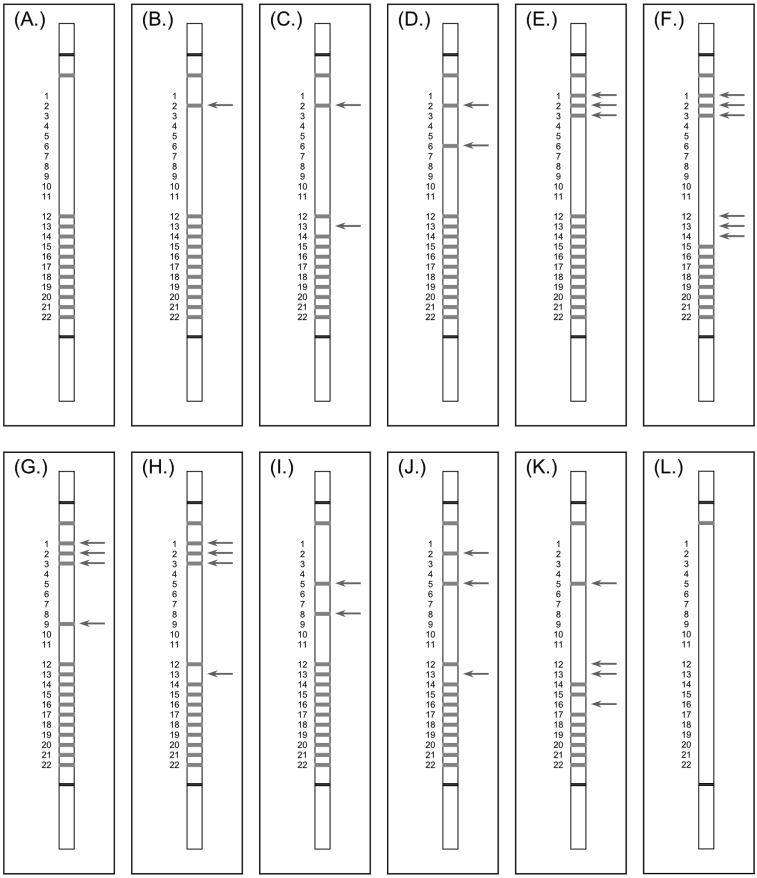

Fig. 3: Examples of results obtained with the CAH StripAssay®

(A.) normal
(B.) I2 splice heterozygous
(C.) I2 splice homozygous or hemizygous
(D.) I2 splice - V282L heterozygous
(E.) P31L - I2 splice - Del 8 bp heterozygous (heterozygous "30 kb E1-E3 deletion")
(F.) P31L - I2 splice - Del 8 bp homozygous (homozygous "30 kb E1-E3 deletion")
(G.) P31L - I2 splice - Del 8 bp - R357W heterozygous (E1-E3 del - R357W)
(H.) P31L - Del 8 bp heterozygous, I2 splice hemizygous (E1-E3 del - I2 splice)
(I.) Cluster E6 - Q319X heterozygous
(J.) Cluster E6 - I2 splice heterozygous
(K.) Cluster E6 homozygous or hemizygous
(L.) E1-E6 homozygous deletion or negative control or PCR failure

XVIII. RELATED PRODUCTS

REF		Σ
7-410	CAH RealFast™ CNV Assay	100 reactions
CS-012	StripAssay® Detection Reagents	20 tests
CS-017	StripAssay® Detection Reagents 48	48 tests
2-014	GENXTRACT™ Blood DNA Extraction System	100 extractions
2-020	Spin Micro DNA Extraction Kit	20 extractions
6-080	Typing Trays	5

Distributor:

Manufacturer:

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